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THE SPECIFICITY OF PROTEINS AND CARBOHYDRATES IN RELATION TO GENERA, SPECIES AND VARIETIES*

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It has long been known that both animals and plants can be definitely grouped upon the basis of the presence or absence of some particular kind of substance or group of allied substances, etc.: animals, in accordance with the presence or absence of haemoglobin, haemocyanin or myogen, etc.; and plants as to whether or not they contain starch, glycogen, tannic acid, some peculiar forms of toxic substances or some particular form of protein, etc. As regards proteins, it has been found that proteins of seeds from different plant sources are not identical and that similar or apparently identical proteins are found only in seeds that are botanically closely related. From the results of a series of elaborate investigations that are being carried on under the auspices of the Carnegie Institution of Washington we may go farther than this gross differentiation of groups and state that a given substance such as haemoglobin or starch may exist in modified forms which in number may infinitely exceed the number of known genera, species and varieties, and that from present indications these modifications are specifically taxonomic.

In order to have clear conceptions of the possibilities of such inconceivably numerous forms of a single substance it is essential that we recall to mind certain salient facts regarding modern conceptions of molecular structure. It will be remembered that when substances have the same kinds and the same number of each kind of atoms they are isomers, and have the same molecular formula; that if isomers so differ in their properties as to indicate that they are different

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substances, the differences are owing to variations in the linkages of the components, which differences are expressed by structural formulae that set forth the linkages in the two dimensions of space; and that when substances have the same molecular and structural formulae but differ in their properties, the differences are due to variations in the arrangements of the components in three dimensions of space, that is, in the configurations of the molecules, which differences are expressed by space formulae. Bodies belonging to the last group are known as stereoisomers or corresponding substances, that is, each kind of substance may exist in a number of forms, all of which forms have the same molecular formula, the same structural formula, and the same fundamental properties in common, but each in accordance with variations in intramolecular configuration has certain individualities which distinguish it from the others.

There are many known substances that exist in stereoisomeric forms, and it has been found that the number of possible forms of each substance is dependent upon the possible number of variations of the arrangements of the molecular components in the three dimensions of space, or, in other words, of variations of molecular configuration, the possible number in case of each substance being capable of mathematical determination. Thus, we find that serum albumin may exist in as many as a thousand million forms. Haemoglobin, the red coloring matter of vertebrate blood, is a far more complex carbon compound than serum albumin, and theoretically may exist in forms whose number is beyond human conception, running into millions of millions. The same is true of starch.

Elsewhere¹ have been set forth with sufficient fullness the hypotheses and theories that underlie an elaborate series of researches which have as their primary object an investigation of corresponding substances obtained from various forms of plant and animal life in relation to taxonomy, sports, mutations, reversions, heredity in general, tumor formation, etc., and nothing more seems necessary in the present address than to state that these researches have as their essential basis the conception that in different organisms the corresponding complex organic substances which constitute the supreme structural components of protoplasm and the major synthetic products of protoplasmic activity are so different as to impart specific peculiarities to the organisms in which they are formed, to be as distinctive of the

¹ Publications 116 and 173 and the Year-Books 9 to 13 of the Carnegie Institution of Washington.

genus or species or sex as the data of the systemist, and to be determinate of the specificity of the protoplasm itself.

It follows from what has been stated that haemoglobin may not only exist in nature in countless forms, but also that each form may be absolutely characteristic of the genus and species.

In an investigation of the haemoglobins it was found that these substances exhibit differences in solubility, decomposibility in relation to putrefactive organisms, quantity of water of crystallization, decomposibility in relation to various chemical reagents, extinction coefficients and quotients, crystallizability, and form and habit of crystallization. The characters of the crystals were especially studied, particularly the forms and habits of crystallization, the peculiarities of twinning, and the "optical reactions," which latter as determined by the aid of the polarizing microscope may be found analytically to be as definite and exact as the reactions obtained by the conventional methods of the chemist. It was found, for instance, in these studies which embraced examinations of specimens of haemoglobins from over 100 species representing many genera and families:

1. That there is a common structure of the haemoglobin molecule whatsoever the source of the haemoglobin.
2. That the crystals of the species of any genus belong to a crystallographic group which represents a generic type.
3. That the crystals of each species of a genus when favorably developed can be distinguished from those of other species of the genus.
4. That the crystals of different generic groups differ as definitely and specifically as those of crystalline groups of mineral substances differ chemically, and as generic groups differ zoologically or botanically.
5. That by means of the peculiarities of haemoglobins phylogenetic relationships can be traced, as has been found in the case of the bear and certain other animals.

Subsequent studies with other substances, especially with animal and plant proteins, a large number of starches, some glycogens and chlorophylls and other complex metabolites, have elicited confirmatory results and even extended the data of the haemoglobin research.

The investigations with the starches were necessarily carried on by methods that are quite different from those employed in the study of the haemoglobins. Although the starch granule is a spherocrystal that lends itself to crystallographic study, very little can be learned

of its molecular characters that is of usefulness in the differentiation of various starches. Other methods, however, offer very satisfactory means of study, especially those which elicit molecular differences by means of peculiarities of gelatinization. These methods, all microscopic, have included inquiries into histological characters, polariscopical, iodine and aniline reactions; temperatures of gelatinization; and quantitative and qualitative gelatinization reactions with a variety of chemical reagents which represent a wide range of difference in molecular composition.

Each starch property, whether it be manifested in peculiarities in size, form, hilum, lamellation or fissuration, or in reactions to light, or in color reactions with iodine or anilines, or in gelatinization reactions with heat or chemical reagents, is an expression of an independent physico-chemical unit-character that is an index of specific peculiarities of intramolecular configuration, the sum of which is in turn an index which expresses specific peculiarities of the constitution of the protoplasm that synthesized the starch molecule. The unit-character represented by the form of the starch grain is independent of that of size; that of lamellation independent of that of fissuration, etc. This is evident in the fact that in different starches variations in one may not be associated with variations in another, and that when variations in different properties are coincidentally observed they may be of like or unlike character. Gelatinizability is one of the most conspicuous properties of starch and it represents a primary physico-chemical unit-character, which character may be studied in as many quantitative and qualitative phases as there are kinds of starches and kinds of gelatinizing reagents, the phenomena of gelatinization by heat being distinguishable from those by a given chemical reagent, and those by one reagent from those by another, and those of one starch by a given reagent from those of another starch. The gelatinization of the starch grain is certainly not, as is commonly supposed, a manifestation of a simple process of imbibition of water, such as occurs in the swelling of particles of dry gelatin or albumin, but in fact a very definite chemical process corresponding to that which occurs in the swelling of liquid crystals, and which must vary in character in accordance with the reagent entering into the reaction. It therefore follows, as a corollary, that the property of gelatinizability of any specimen of starch may be expressed in as many independent physico-chemical unit-character-phases as there are reagents to elicit them.

By these methods physico-chemical unit-characters and unit-character-phases can be reduced to figures from which charts can be constructed which show in the case of each starch that the sum-total of these values is as distinctive of the kind of starch and plant source as are botanical characters of the plant. In determining these values certain precautions must sedulously be observed in order to obtain dependable results. Thus, in the polarization, iodine and aniline reactions, definite though arbitrary standards of comparison must be adopted. This can crudely but satisfactorily be accomplished by selecting as standards three or four starches which exhibit desired gradations of value, and constructing a scale by the aid of which values can be reduced in figures. In all of the gelatinization reactions the examinations must be made on the stage of the polarizing microscope. In determining the temperatures of gelatinization especial care must be exercised in regard to uniformity in the rapidity with which the preparations are heated, together with such other precautions as have been found essential in determining "melting points." In the reactions with the chemical reagents it is absolutely essential that immediately upon the addition of the reagent to the starch on the slide the preparation be kept air-tight to avoid changes in concentration of the reagent by loss or addition of water, and to avoid effects of oxidation. It goes without saying that in the iodine, aniline and chemical-reagent experiments it is necessary to use definite and constant proportions of reagent and starch. Finally, inasmuch as the starch of any given plant varies somewhat in relation to season, rest and activity, the part of the plant in which it is formed, etc., it obviously is important in comparative experiments such as those under consideration to obtain specimens from corresponding parts of plants and under other corresponding conditions. In the present researches all of the starches were prepared from bulbs, tubers, rhizomes, etc., in the resting state. Each preparation was obtained from a number of specimens, usually 25 to 50, so that each is representative of the plant source.

The measure of value to be attached to the results of researches that are carried out along such exceptional lines, and by means of such seemingly gross methods of study, must naturally rest inherently upon the uniformity of the results of repeated experiments and upon the conformity of the results with established data of the systematist. As to the former, it need only be stated that when experiments have

been repeated, even though under varying laboratory conditions as regards temperature and humidity, the results have been either identical or have differed so little as to be absolutely unimportant. As to the latter, it will be found that the records are to an astonishing degree in harmony with established botanical peculiarities, and that where perchance there may be departure the causes therefor are usually not far to seek. It perhaps is needless to state that as great care and technical skill may be required to conduct successfully such experiments as are demanded where the methods are in the conventional sense exact, and consequently that much training may be necessary before one is fitted to make permanent records.

In these researches it has been found desirable to record the quantitative gelatinization values in three kinds of charts, each presenting in its own particular and impressive way certain striking peculiarities which are not at all or not so well exhibited by another. One kind shows the progress of gelatinization in time-percent reaction curves of the starch with a given reagent; another, the differences in gelatinizability of different starches with a given reagent; and another, composite curves of reaction-intensities of a given starch with a number of agents and reagents, by means of which types of curves of varieties, species and genera are obtained.

From these investigations the following fundamental statements are deduced:

1. The results of the haemoglobin and starch researches are mutually confirmatory in proving the existence of different stereoisomeric forms that are specifically modified in relation to varieties, species and genera, in other words, stereoisomeric specificity in relation to taxonomy; and that such specificities indicate corresponding specificities of the protoplasms that give rise to these different forms.
2. The reaction-intensities of different starches with a given reagent vary within wide limits, and vary with each reagent independently of the variations of other starches.
3. The reaction-intensities of varieties of a species very closely correspond with those of the species and they are in accord with botanical characters.
4. The reactions of different species of a genus exhibit characters in conformity in general with the values of the distinguishing botanical characteristics of the members of the genus, the species-characters varying in degree of closeness or separation in accord with corresponding botanical peculiarities.

5. The reactions of the members of a genus constitute a well-defined group, the mean of the character-values constituting a distinct generic type.

6. When a genus consists of subgenera, or groups of rhizomatous and tuberous plants, or tender and hardy plants, etc., there may be as many subgeneric types as there are groups.

7. When subgeneric types exist they may be bridged in part by intermediate characters of a hybrid that is the offspring of members of such types.

8. The generic types belonging to a given family tend in general to exhibit closeness or separation in accord with established botanical data.

These researches have been of a purely exploratory character. No attempt has been made to establish by sufficiently repeated experiments and otherwise what may be accepted as character-constants, but rather to establish certain principles and a foundation or starting point for final investigation. In fact, there remains much to be done in the way of preliminary work before the final studies are begun. Particularly important is it to extend the period of observation, modify the concentrations of some of the reagents, introduce additional reagents, improve the polarization and the iodine and aniline methods or eliminate them, and study the stereoisomers of successive generations in relation to various conditions that influence heredity. Considerable advances must be made in order to demonstrate satisfactorily certain taxonomic differences that undoubtedly exist, and also to set forth satisfactorily these differences in the form of composite charts. In such composite charts as constructed up to the present no difference may be shown between two starches in a given reaction, yet the records may show that during the progress of gelatinization more or less marked differences were recorded. Hence, in taxonomic studies it is essential to consider collectively all three kinds of charts, and in association with the histological characters and qualitative reactions. In other words, in the ultimate analyses and comparisons we must utilize the sum-total of characters and character-phases.

In conclusion, it need scarcely be stated that limitations of time have made the presentation of so extensive a topic of a somewhat scrappy character, and that notwithstanding the incompleteness and various defects of these researches facts and principles of epochal

importance in relation to the mechanisms of living matter have been brought to light, and hence the way shown for developments of the greatest moment in normal and abnormal biology, as for instance:

1. It seems obvious that we have found a strictly scientific basis for the classification of plants and animals.

2. There are manifestly certain striking applications that are of the greatest fundamental importance in the study of phylogeny, mutations, reversions, sex, malformations, phenomena of heredity in general, etc. (For an application, see article on *The Germplasm as a Stereochemic System*, *Science*, **40**: 649-661. 1914.)

3. The discovery of the existence of highly specialized stereoisomers has brought before us one of the most remarkable and unsuspected phenomena of living matter, and one which leads us directly to the constitutions of various forms of protoplasm and the peculiarities of vital phenomena that are dependent upon these differences.

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